

2. Notes on an *Ichthyosporidian* causing a Fatal Disease in Sea-Trout. By MURIEL ROBERTSON, Carnegie Research Fellow *.

[Received March 1, 1909.]

(Plates LXII.-LXIV.†)

In July 1907 Dr. Turnbull, of the London Hospital Medical School, while fishing on the River Ewe in Ross-shire, observed that a number of sea-trout were affected with a disease which culminated in the death of the fish. Externally they showed nothing abnormal, but on opening them he found that the heart, liver, spleen, and pyloric caeca showed minute sand-like granules.

Dr. Turnbull's description of a typical infection reads as follows:—

"The gills were healthy and showed no adherent parasites.

"The heart: the ventricle was pale, rough and sandy, and minute white granules could be seen. Similar granules were on the auricles and sinus venosus.

"The liver was of a yellow colour and showed minute, yellowish-white, slightly raised, rounded granules on the surface and in section.

"In the spleen very few granules could be detected.

"The alimentary tract was empty and no duodenal tape-worms were present.

"The brain, on removal, showed no abnormalities.

"The ovaries showed no granules."

Some fish, however, showed an even more complete infection than this; thus in some cases the pyloric caeca, the gills, the submucosa of the oesophagus, the testicles, and the parietal muscles beneath the peritoneum all showed the characteristic sanded appearance.

Dr. Turnbull observed that the diseased sea-trout were all fresh-run fish and were generally of about 4 lbs. weight and over, although one infected fish was got which weighed 2 lbs.

The River Ewe runs from "Loch Maree" into the sea-loch "Loch Ewe" at "Poolewe." It is a short river, only about a mile and half long.

The various internal organs of some of the infected fish were preserved in methylated spirit, this being the only preserving-fluid obtainable. Sections were made which were sent along with Dr. Turnbull's notes to Prof. Minchin at the Lister Institute. The disease proved to be caused by a Protozoan parasite belonging to the genus *Ichthyosporidium*.

In January 1909 Prof. Minchin handed over to me the material together with his own and Dr. Turnbull's notes, and it

* Communicated by Prof. E. A. MINCHIN, M.A., V.P.Z.S.

† For explanation of the Plates see page 402.

is from these that this account of the parasite has to a large extent been drawn.

The genus *Ichthyosporidium* belongs to the family of the Bertramiidae and to the order of the Haplosporidia. It was created by Caullery and Mesnil in 1905 for the two parasites *Ichthyosporidium gasterophilum* and *Ichthyosporidium phymogenes**.

Ichthyosporidium gasterophilum, which is closely allied to if not identical with the parasite in the sea-trout, is found in *Motella mustela* and *Liparis vulgaris*.

These are both shore-living fishes and inhabit the Fucus-belt. Caullery and Mesnil note that *Motella mustela* (the rockling) was most frequently infected and that the infected fish were all above a certain size. The parasite was found only in the pyloric caeca and the ducts of the glands of the stomach. The authors do not mention if the presence of the *Ichthyosporidium* seriously affected the health of the fish.

The *Ichthyosporidium* of the sea-trout can easily be recognised in sections of the tissues as large spherical or elongated bodies (figs. 1-8). The material only shows the trophical stages of the parasite and the fixation is not quite without reproach.

The parasites are multinucleate organisms, most often spherical in shape and possessing a well-marked outer envelope. They range from large individuals with many hundred nuclei, measuring 120 μ m. in diameter, to small forms with a thin envelope and few nuclei. Individuals with only two nuclei are to be found and some apparently with a single nucleus, but it is difficult to be quite certain of these. They are very rare, and in section-material of this type an error in such a point is very easily made.

The envelope is secreted by the parasite; the inner part is smooth and structureless except for occasional striations (figs. 6 & 7). The outer part of the envelope is often slightly crinkled. The shape of the organism is open to very considerable variation; oval, vermiform, and even irregularly branched forms may be found. These are for the most part individuals which have quitted their original envelope and grown out into the tissues. They seem to secrete a secondary envelope, which is generally rather thin; moreover, this may not extend over the whole animal.

Sometimes these large irregular specimens seem to be breaking up into several daughter individuals by the very simple process of plasmotomy. The products of this process are of very varying sizes. It is a very casual method and does not involve nuclear division—it is, in fact, merely the breaking off of a mass of nucleated protoplasm.

* Caullery and Mesnil, C. R. Soc. Biol. Paris, lviii. (1905) pp. 640-642.

It is impossible to say exactly what brings about the exit from the envelope. It is not a question of size, as quite small specimens may be found fixed at what appears to be the moment of escape.

The nuclei in *Ichthyosporidium* are, relatively speaking, very small and very numerous. They consist each of a single compact mass of chromatin surrounded by a clear space which is bounded by a delicate sharply-defined membrane. Very fine rays pass from the mass of chromatin to the membrane. The mass of chromatin or karyosome is dense and compact, and shows no structure, no matter what the stain used. Iron-haematoxylin, Delafield's haematoxylin, carm-alum and picronigrosin, picrocarmine and picronigrosin, and Twort's neutral red and light green stain were all tried and all gave very fair results. Iron-haematoxylin and Twort's stain, however, gave the best pictures. The rays passing from the karyosome to the outer membrane are exceedingly fine and stain rather faintly with the chromatin stains. The membrane takes up the chromatin colours faintly when stained with iron-haematoxylin, Delafield's haematoxylin, &c., but takes up the green of the Twort's stain—that is to say, it does not take the chromatin stain of this combination. This is a rather interesting point, as it indicates that whatever its nature it is not the mere condensation of chromatin which generally constitutes the so-called "membrane" in the Protozoa. It may be noted that the red colour in Twort's stain, when properly applied, appears to be a pretty good test for chromatin.

No stages showing nuclear division were observed nor was there any indication of spore-formation.

As has already been stated, the parasite invades practically all the organs of the trout, but seems always to be found in the greatest numbers in the muscles of the heart.

The reaction on the part of the host in the way of forming connective-tissue cysts round the parasites is comparatively slight. In the liver and spleen, it is true, cysts composed of many layers of connective-tissue cells are formed, especially in the case of the larger parasites; but in other parts of the body, such as the heart-muscles, the cysts consist only of two or three thin layers of cells, and may sometimes not be formed at all. The envelope secreted by the parasite in these cases lies in direct contact with the striped muscle-fibres. This relatively slight development of connective tissue may have a certain importance in explaining the fatal nature of the disease.

Three years ago, in January 1906, I came across an *Ichthyosporidium** identical, as far as I can see, with the one found in the trout, in a small flounder (*Pleuronectes flesus*).

The fish was also infected with a trypanosome, which was the parasite I was in search of, and I unfortunately did not examine

* Proc. Roy. Phys. Soc. of Edinburgh, vol. xvii. No. 5, 1908.

the heart. The liver and the submucosa of the stomach and the intestine were very strongly infected, far more so than any of the organs from the trout. This material gave a more complete picture of the trophic life of the parasite. Such points as the multiplication of the nuclei, the exit from the envelope, and the breaking up of the large individuals into numerous small naked bodies with few nuclei were clearly illustrated in the sections. There were, however, no signs of sporogony. In the flounder very large connective-tissue cysts were formed both in the liver and in the alimentary canal. The only difference between the infection in the sea-trout and that in the flounder is in the greater development of connective tissue on the part of the latter host. The flounder in question must ultimately, I should think, have succumbed to the disease, as the liver was in a very pathological condition, but it had been living for some months in captivity in the tanks of the Millport Marine Station and showed no external sign of ill-health.

From the material at my disposal only a very incomplete account of the *Ichthyosporidium* can be given. It is to be hoped that this form, which is interesting both from a purely scientific as well as an economic point of view, will receive further attention from Protozoologists and will be studied under more favourable conditions.

Since writing the above paper another case of the occurrence of this disease has come under my notice in haddocks from Aberdeen. A fishmonger in Glasgow, while cutting the fish into filets, observed minute yellowish white granules in the tissues. He sent the filets to Prof. Graham Kerr, who forwarded them to me at the Lister Institute. It was found that the appearance was due to the presence of an immense number of an *Ichthyosporidian* apparently identical with the one described in this paper.

EXPLANATION OF PLATES LXII.-LXIV.

Ichthyosporidians of Sea-Trout.

- Fig. 1. Magn. of 35 diam. Showing general appearance of the parasites in section of the heart.
 2. Magn. of 100 diam. General view of the parasites, showing different shapes assumed.
 3. Magn. of 250 diam. Single large parasite, showing the envelope.
 4. Magn. of 250 diam. Single large parasite; the shrinkage is due to the fixation, as also the appearance of the nuclei at one side. The envelope shows clearly.
 5. Magn. of 500 diam. Shows thin layer of connective tissue round the parasite; envelope of the *Ichthyosporidium* and the nuclei are both shown.
 Figs. 6 & 7. Magn. of 1000 diam. These two figures show the nuclei; the fine rays passing from the karyosome to the membrane can be seen.
 Fig. 8. Magn. of 1000 diam. Shows the nuclei distorted in the process of fixing.
 9. Magn. of 750 diam. Gives another appearance caused by fixation.

These figures are from untouched photographs kindly executed by Dr. Reid.